PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
G01N 33/68, 33/50, C12Q 1/48

A1

(11) International Publication Number: **WO 99/58982**

(43) International Publication Date: 18 November 1999 (18.11.99)

(21) International Application Number: PCT/US99/10416

(22) International Filing Date: 12 May 1999 (12.05.99)

(30) Priority Data:

60/085,439	14 May 1998 (14.05.98)	US
09/156,367	17 September 1998 (17.09.98)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	09/156,367 (CON)
Filed on	17 September 1998 (17.09.98)
US	60/085,439 (CON)
Filed on	14 May 1998 (14.05.98)

(71)(72) Applicant and Inventor: LIU, Ya, Fang [CA/US]; One Emerson Place 5G, Boston, MA 02114 (US).

(74) Agents: CARROLL, Alice, O. et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02421 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD TO IDENTIFY COMPOUNDS TO PREVENT NEURON DEATH

(57) Abstract

The present invention describes methods for identifying compounds that inhibit JNK and MLK kinase activity as drugs for treating a mammal susceptible to or having a neurological condition. This invention also discloses methods for preventing neuronal cell death and treating neurological conditions that involve neuronal cell death, particularly neurodegenerative diseases characterized by glutamine or kainate mediated toxicity, such as Huntington's disease and Alzheimer's disease.

CLAIMS

What is claimed is:

1. A method for assessing a compound's ability to prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition, comprising:

   a) contacting a compound with neuronal cells having activated MLK and/or JNK activity;

   b) determining the number of neuronal cells that die;

   wherein a decreased number of dead neuronal cells in the presence of the compound compared to the number of dead neuronal cells in the absence of the compound is indicative of the compound's ability to prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition.

2. The method of Claim 1, wherein the neuronal cells are transfected with a mutated protein or treated with a neurotoxin to induce apoptosis.

3. The method of Claim 2, wherein the neuronal cells are HN33 cells.

4. The method of Claim 2, wherein the mutated protein is polyglutamine stretch-expanded huntingtin or C-terminal 100 amino acids of amyloid precursor protein.

5. The method of Claim 2, wherein the neurotoxin is glutamate, quinolinic acid or kainic acid.

6. The method of Claim 1, wherein the neuronal cells are apoptotic neurons.

7. The method of Claim 1, wherein the neurological condition is a neurological disease whereby glutamate or kainic acid mediated excitotoxicity is involved in neuronal cell death.

8. The method of Claim 1, wherein the neurological condition is Huntington's disease or Alzheimer's disease.

9. A method for assessing a compound's ability to prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition, comprising:

a) contacting a compound with neuronal cells transfected with a mutated protein or treated with a neurotoxin that induces neuronal cell death; and

b) determining the number of neuronal cells that die;

wherein a decreased number of dead neuronal cells in the presence of the compound compared to the number of dead neuronal cells in the absence of the compound is indicative of the compound's ability to prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition.

10. The method of Claim 9, wherein the neuronal cells are HN33 cells.

11. The method of Claim 9, wherein the mutated protein is polyglutamine stretch-expanded huntingtin or C-terminal 100 amino acids of amyloid precursor protein.

12. The method of Claim 9, wherein the neurological condition is a neurological disease whereby glutamate or kainic acid mediated excitotoxicity is involved in neuronal cell death.

13. The method of Claim 9, wherein the neurological condition is Huntington's disease or Alzheimer's disease.

14. A method for assessing the ability of a JNK and/or MLK inhibitor to prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition, comprising:

a) contacting a JNK and/or MLK inhibitor with neuronal cells having activated MLK and/or JNK activity;

b) contacting, in the presence of the compound, surviving cells from step (a) with an agent that induces apoptosis; and

c) comparing the level of apoptosis in the cell in the presence of the JNK and/or MLK inhibitor with the level of apoptosis in the cell in the absence of the JNK and/or MLK inhibitor;

wherein the JNK and/or MLK inhibitor is a potentially useful drug for treating the mammal when the level of apoptosis in the cell in the presence of the JNK and/or MLK inhibitor is less than the level of apoptosis in the cell in the absence of the JNK and/or MLK inhibitor.

15. The method of Claim 14, wherein the apoptotic agent is a neurotoxin.

16. The method of Claim 14, wherein the neurotoxin is glutamate, quinolinic acid or kainic acid.

17. The method of Claim 14, wherein step (b) is performed by transfecting the surviving neuronal cells with a mutated form of huntingtin or amyloid precursor protein.

18. The method of Claim 14, wherein the neuronal cells are HN33 cells.

19. A method for screening a compound's ability to inhibit JNK and/or MLK activity and thereby prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition, comprising:

a) contacting a compound with a JNK and/or MLK protein and substrate therefor;

b) measuring the level of JNK and/or MLK activity;

c) comparing the level of JNK and/or MLK activity in the presence of the compound with the level of JNK and/or MLK activity in the absence of the compound, wherein a decrease in JNK and/or MLK activity in the presence of the compound is indicative that the compound is a JNK and/or MLK inhibitor;

d) contacting the compound with neuronal cells having activated MLK and/or JNK activity;

e) comparing the occurrence of apoptosis in the cell in the presence of the compound with the occurrence of apoptosis in the cell in the absence of the JNK and/or MLK inhibitor;

wherein the JNK and/or MLK inhibitor is a potentially useful drug for treating the mammal when the occurrence of apoptosis in the cell in the presence of the JNK and/or MLK inhibitor is less than the occurrence of apoptosis in the cell in the absence of the JNK and/or MLK inhibitor.

20. The method of Claim 19, wherein JNK is JNK1, JNK2 or JNK3 and MLK is MLK1, MLK2 or MLK3, or combinations thereof.

21. The method of Claim 20, wherein the JNK and/or MLK activity is a kinase activity.

22. The method of Claim 19, wherein the neurological condition is a neurological disease whereby glutamate or kainic acid mediated excitotoxicity is involved in neuronal cell death.

23. The method of Claim 19, wherein the neurological condition is Huntington's disease or Alzheimer's disease.

24. A method for assessing a compound's ability to inhibit JNK and/or MLK activity and thereby prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition, comprising:

a) incubating a compound in the presence of JNK and/or MLK and appropriate JNK and/or MLK substrate therefor, under conditions sufficient for enzymatic activity; and

b) determining the presence or amount of phosphorylated product;

wherein a change in amount of phosphorylated product, when compared to incubating JNK and/or MLK with appropriate substrates absent the compound, is indicative of the compound's ability to inhibit the enzymatic activity of JNK and/or MLK and thereby prevent neuronal cell death in a mammal susceptible to or having a neurological condition.

25. The method of Claim 24, wherein JNK is JNK1, JNK2 or JNK3 and MLK is MLK1, MLK2 or MLK3, or combinations thereof.

26. The method of Claim 24, wherein JNK substrates include c-Jun and a phosphate donor.

27. The method of Claim 24, wherein phosphorylated product of step (b) is phosphorylated c-Jun or phosphorylated SEK1.

28. The method of Claim 27, wherein the MLK substrates include SEK1 and a phosphate donor.

29. A method for assessing a compound's ability to inhibit JNK and/or MLK kinase activity and thereby prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition, comprising:

(a) contacting a neuronal cell with a compound under conditions sufficient for JNK and/or MLK enzymatic activity; and

(b) determining the presence or amount of phosphorylated JNK and/or MLK product;

wherein a change in amount of phosphorylated product, when compared to incubating a cell absent the compound, is indicative of the compound's ability to inhibit JNK and/or MLK kinase activity and thereby prevent neuronal cell

death occurring in a mammal susceptible to or having a neurological condition.

30. The method of Claim 29 further comprising:

(c) determining cell viability after step (a);

wherein any increase in the cell's viability status relative to a control is indicative of the compound's ability to inhibit JNK and/or MLK kinase activity thereby affecting the viability of the cell.

31. The method of Claim 29, wherein alteration in the cell's viability status includes apoptosis.

32. The method of Claim 29, wherein JNK is JNK1, JNK2 or JNK3 and MLK is MLK1, MLK2 or MLK3, or combinations thereof.

33. A method for assessing a compound's ability to inhibit MLK and/or JNK kinase activity and thereby prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition, comprising:

(a) administering to an animal an amount of compound under conditions sufficient to allow for proper pharmacodynamic absorption and distribution thereof in the animal; and

(b) determining the physiological status of the animal;

wherein a change in physiological status, when compared to an animal not administered the compound, is indicative of the compound's ability to inhibit MLK and/or JNK kinase activity and thereby prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition.

34. The method of Claim 33, wherein MLK is MLK1, MLK2 or MLK3 and JNK is JNK1, JNK2 or JNK3, or combinations thereof.

35. The method of Claim 33, wherein the animal is a mammal.

36. A method for treating a neurological disorder in a mammal in need thereof, comprising administering to the mammal an effective therapeutic amount of a compound that inhibits JNK and/or MLK activity and thereby prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition.

37. The method of Claim 36, wherein the neurological condition is a neurological disease whereby glutamate or kainic acid mediated excitotoxicity is involved in neuronal cell death.

38. The method of Claim 36, wherein the neurological condition is Huntington's disease or Alzheimer's disease.

39. The method of Claim 36, wherein JNK is JNK1, JNK2 or JNK3 and MLK is MLK1, MLK2 or MLK3, or combinations thereof.

40. A method for preventing neuronal cell death in a mammal susceptible to or having a neurological condition, comprising administering to the mammal in need thereof an effective therapeutic amount of a compound that inhibits JNK and/or MLK activity in a neuronal cell and thereby prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition.

41. The method of Claim 40, wherein the neurological condition is a neurological disease whereby glutamate or kainic acid mediated excitotoxicity is involved in neuronal cell death.

42. The method of Claim 40, wherein the neurological condition is Huntington's disease or Alzheimer's disease.

43. A method for treating a neurological disorder in a mammal in need thereof, comprising administering to the mammal an effective therapeutic amount of a compound that inhibits JNK and/or MLK activity and thereby prevent

neuronal cell death occurring in a mammal susceptible to or having a neurological condition, wherein the compound is identified by a method for assessing a compound's ability to prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition, comprising:

a) contacting a compound with neuronal cells having activated MLK and/or JNK activity;

b) determining the number of neuronal cells that die;

wherein a decreased number of dead neuronal cells in the presence of the compound compared to the number of dead neuronal cells in the absence of the compound is indicative of the compound's ability to prevent neuronal cell death occurring in a mammal susceptible to or having a neurological condition.